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10275-045002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : DiTullio et al.
Serial No. : 09/143,155
Filed : August 28, 1998
Title : TRANSGENICALLY PRODUCED ANTIHTROMBIN III

Art Unit : 1632
Examiner : G. Lee

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF CAROL ZIOMEK, Ph.D., UNDER 37 CFR 1.132

Sir:

I, Carol Ziomek, Ph.D., hereby declare and state as follows:

1. My educational and professional experience is presented in the attached Curriculum Vitae (Appendix A).

reconsidered Ziomek 2/19/02

2. I am employed by Genzyme Transgenics Corporation, the assignee of the above-identified application.

3(a). I have been advised and understand that the Examiner has rejected the claims of the above-identified application. The claims are directed to mammary gland produced antithrombin III having a monosaccharide composition which differs from plasma derived antithrombin III. The claims are limited to antithrombin III, having one or more of the following distinguishing characteristics: the presence of GalNAc (plasma derived antithrombin III lacks GalNAc); the presence of fucose (plasma derived antithrombin III is not fucosyleted); and/or having a higher level of oligomannose or hybrid mannose structure than does plasma derived antithrombin III. I have further been advised and understand that this rejection is based on the Examiner's assertion that the specification does not provide guidance regarding transgenic mammals other than goat which would produce antithrombin III with the claimed monosaccharide composition.

3(b). Applicants have shown that the claimed glycosylation patterns occur in mammary gland produced antithrombin III of very divergent species, namely goat and mouse. Thus, applicant has enabled a broad claim to mammary production in mammals. Further, applicant has shown, by way of a number of comparisons that the differences are organ and not species specific. The substitution of GalNAc for galactose is the function

of expressing ATIII in the mammary gland and not a species difference particular to goat. It was also disclosed that the presence of fucose and the higher levels of oligomannose and hybrid mannose structures are found in mammary gland produced antithrombin III as compared to plasma derived antithrombin III. This has been further evidenced by the results provided herein. Since Applicants describe a transgene which encodes ATIII and is expressed in the mammary gland of different species of transgenic mammals, and ATIII produced in the mammary tissue has the claimed monosaccharide compositions, there is clearly sufficient guidance to make and use the claimed mammary gland produced ATIII.

4. As is discussed in the specification, both transgenic goats and transgenic mice have been produced using the same transgene (i.e., the B6C transgene). Both the transgenic goats and the transgenic mice express ATIII having the claimed monosaccharide compositions in their mammary tissue. In particular, at page 8, lines 4-22 of the specification, transgenic mice were produced by microinjection of the B6C transgene which is the same transgene used to produce the transgenic goats described in the present application.

5. As discussed in the specification, antithrombin III was expressed at levels of up to 0.7 to 1.0 mg/ml in the milk of such transgenic mice. Using the same B6C transgene, transgenic goats were also produced which expressed ATIII at levels up to 4 to 6 mg/ml in their milk.

6 (a). In order to determine if the claimed monosaccharide compositions of antithrombin III were mammary gland specific, we have analyzed the monosaccharide structures of antithrombin III produced in the mammary gland of transgenic mice and transgenic goats. We have also compared the monosaccharide composition of these mammary gland produced antithrombin III with the monosaccharide composition of plasma-derived antithrombin III from two species, humans and goats. Our work shows the following.

6 (b). Comparison of the monosaccharide composition of mammary gland produced antithrombin III from transgenic goats and transgenic mice show that the claimed monosaccharide compositions of mammary gland produced antithrombin III are

not species specific. ATIII from the mammary glands of these two highly divergent species shared the same modifications, namely, those discussed in paragraph 3.

6 (c). Comparison of the mammary gland produced antithrombin III to plasma derived antithrombin III provides evidence that the claimed monosaccharide compositions of mammary gland produced antithrombin III differ from plasma derived antithrombin III.

6 (d). Finally, comparison of the monosaccharide composition of mammary gland produced antithrombin III from transgenic goats to plasma derived antithrombin III from goats provides evidence that monosacharide characteristics are organ specific not species specific.

These results are discussed in more detail below.

7. Transgenic mammary produced antithrombin III from goats contains GalNAc and thereby differs from human plasma-derived antithrombin III which contains galactose as opposed to GalNAc. The difference is due to the organ in which the antithrombin III is produced and not due to the species. This is shown by the following. In Cole et al. (1994) *J. Cellular Biochemistry Suppl.* Vol. 0 (18D) p 265, it was found that antithrombin III derived from the plasma of a goat lacked GalNAc and thereby differed from antithrombin III produced in the milk of a transgenic goat which includes GalNAc. Therefore, the substitution of GalNAc for galactose is not a species difference between human antithrombin III and goat antithrombin III. If the effect had been species specific, the substitution of GalNAc for galactose would have been seen in both goat mammary gland produced and goat plasma derived antithrombin III. Instead, the presence of this substitution appears only in mammary gland produced antithrombin III, and thus appears to be organ, i.e., mammary gland, specific.

8. Another difference between antithrombin III made in the mammary gland and antithrombin III derived from plasma is the level of fucosylation of mammary gland produced antithrombin III. Our experiments, the results of which are provided below, show that mammary antithrombin III is heavily fucosylated. We have performed experiments comparing the level of fucosylation of human plasma derived antithrombin III, goat plasma derived antithrombin III, goat mammary gland produced antithrombin III and mouse mammary gland produced antithrombin III. The percent of fucose present in these antithrombin III samples are as follows:

Human Plasma ATIII	Goat Plasma ATIII	Goat Mammary Produced ATIII	Mouse Mammary Produced ATIII
0 %	0 %	100% at Asn^{192} 100% at Asn^{96}	85% at Asn^{192} 85% at Asn^{96}

These results demonstrate that antithrombin III derived from plasma differed from antithrombin III produced in milk and that antithrombin III produced in the milk of two very different mammals had the claimed condition effect. The results also demonstrate that antithrombin III derived from the plasma of a goat differed from antithrombin III produced in the milk of a goat. Therefore, the presence of fucose is not a species difference between human antithrombin III and goat antithrombin III. If the effect had been species specific, the level of fucose would have been the same or similar in both goat mammary gland produced and goat plasma derived antithrombin III. Instead, the presence of fucose is much greater in mammary gland produced antithrombin III, and thus appears to be organ, i.e., mammary gland, specific.

9. The level of oligomannose and hybrid mannose structures is also a conditioned effect of producing antithrombin III in the mammary gland. Experiments have been performed comparing the level of oligomannose and hybrid mannose structures in human plasma derived antithrombin III, goat mammary gland produced antithrombin III and mouse mammary gland produced antithrombin III. The average levels in each of these samples were as follows:

Human Plasma ATIII	Goat Mammary Produced ATIII	Mouse Mammary Produced ATIII
17.19 nMoles	33.80 nMoles	37.8 nMoles

These results demonstrate a higher level of oligomannose and hybrid mannose structures present in antithrombin III produced in milk and antithrombin III derived from plasma. These results also demonstrate the presence of this characteristic in antithrombin III produced in the mammary gland of two very different species, goat and mouse.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/2/01
Date

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